

Array/PCR Training Set

- Simultaneously assay subjects with
 - microarray on frozen samples (20k genes)
 - qRT-PCR on FFPE samples (160 genes)
- Objectively identify gold standard sample classifications using microarray
 - Cluster analysis of intrinsic genes
 - SigClust to identify significant clusters

SigClust: Liu et al., JASA 2008

Array/PCR Training Set

- Construct classification model in qRT-PCR (FFPE) data using gold standard labels
 - Calculate each subtype centroid
 - Compare a test case to each centroid
 - Assign the label of the most similar centroid
 - Repeatedly test with cross-validation over decreasing number of genes

3 methods for ranking genes
3 methods for constructing centroids

Dudoit & Fridlyand JASA 2002
Storey et al. Bioinformatics 2006
Tibshirani et al. PNAS 2002

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 - Select reasonable gene set based on cross-validated accuracy

Final classifier consists of 50 gene centroids from gold standard samples
Accuracy estimate of 93% (91.7-93.6%)

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Validation in Independent Datasets

Table 1. Clinical Characteristics by Cohort

Characteristic	Training set	No adjuvant systemic therapy*	Neoadjuvant chemotherapy*
Samples	189	761	133
Median Followup (yrs)	4	9	18
Mean Age	58+/15	53+/13	52+/11
ER			
+	114	544	82
-	77	195	51
N			
+	96	35	93
-	100	710	40
HER2			
+	NA	66	33
-	NA	192	99
Tumor Size			
<= 2 cm	63	409	13
> 2 cm	136	339	120
Grade			
Low	12	133	2
Med	56	218	54
High	127	286	75
Subtype			
HER2-enriched	31	120	29
Basal-like	56	128	27
Normal-like	12	76	13

*compiled from Ishina et al., 2006; Loi et al., 2007; van de Vijver et al., 2002; Wang et al., 2005; <https://genome.unc.edu/pubsup/breastGEO/>
*Hess et al., 2006

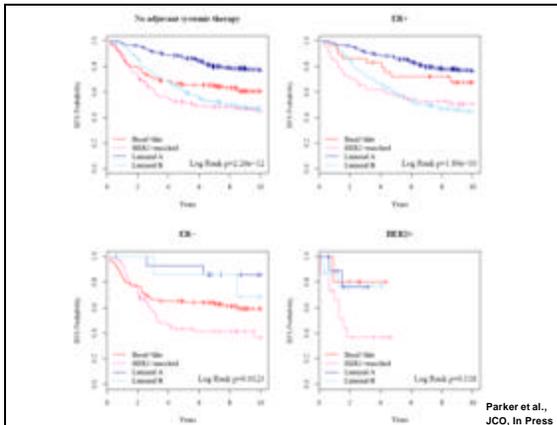
Validation using Test Datasets

Table 1. Clinical Characteristics by Cohort

Characteristic	Training set	No adjuvant systemic therapy*	Neoadjuvant chemotherapy*
Samples	189	761	133
Median Followup (yrs)	4	9	NA
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<= 2 cm	63	409	13
> 2 cm	136	339	120
Grade			
Low	12	133	2
Med	56	218	54
High	127	286	75
Subtype			
Luminal A	23	269	37
Luminal B	12	168	27
HER2-enriched	31	120	29
Basal-like	56	128	27
Normal-like	12	76	13

*compiled from Ishina et al., 2006; Loi et al., 2007; van de Vijver et al., 2002; Wang et al., 2005; <https://genome.unc.edu/pubsup/breastGEO/>
*Hess et al., 2006

Parker et al., JCO, In Press



Prognostic Model Evaluation

Table 2. Models of Relapse Free Survival (untreated)

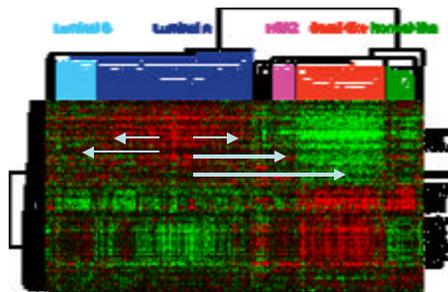
Model	A		B		C	
Variable	Hazard Ratio	p-value	Hazard Ratio	p-value	Hazard Ratio	p-value
Basal-like*	1.33	0.330	1.79	0.030	1.58	0.066
HER2-enriched*	2.53	<0.00012	3.25	<0.0001	2.90	<0.0001
Luminal B*	2.43	<0.0001	2.88	<0.0001	2.54	<0.0001
ER Status-	0.83	0.38	0.83	0.34	0.83	0.32
Tumor Size†	1.36	0.034	1.43	0.012	1.57	0.001
Node Status‡	1.75	0.035	1.72	0.041	-	-
Histologic Grade§	1.40	0.0042	-	-	-	-
Full vs Subtype*	-	<0.0001	-	<0.0001	-	<0.0001
Full vs Clinical¶	-	<0.0001	-	<0.0001	-	<0.0001

*Luminal A class used as reference state in multivariate
-Hazard ratios for ER using positive marker in the numerator
†Size <= 2cm versus >2cm
‡Any positive node
§Grade encoded as an ordinal variable with three levels
¶Significant p-values indicate improved prediction relative to subtype alone
§Significant p-values indicate improved prediction relative to clinical data alone

N=710 local therapy only test cases

Parker et al., JCO, In Press

Diversity Within Subtypes



Distance to each centroid for a genomic summary

Parker et al., JCO, In Press

Risk Classification

- Similarity to the subtypes are used as variables in the prognostic model where the outcome is risk of recurrence (ROR):

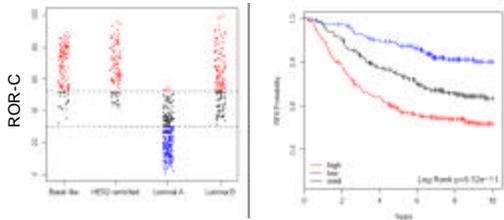
$$(1) ROR_1 = \beta_1 \cdot \text{Basal} + \beta_2 \cdot \text{HER2} + \beta_3 \cdot \text{LumA} + \beta_4 \cdot \text{LumB}$$

$$(2) ROR_2 = \beta_1 \cdot \text{Basal} + \beta_2 \cdot \text{HER2} + \beta_3 \cdot \text{LumA} + \beta_4 \cdot \text{LumB} + \beta_5 \cdot T$$

- Weights for each term are learned from training data using a Cox model with Ridge Regression
- The weighted sum is assigned as the risk score for a test case and a threshold may be applied for class assignment

Ridge regression with Cox model; Tibshirani, Statistics in Medicine 1997
Comparative study; Bovelstad et al. Bioinformatics 2007

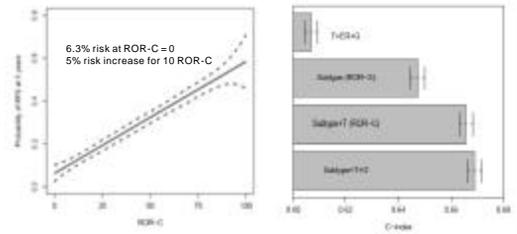
Risk Classification



N=558 untreated test cases

ROR-C threshold determined from training data

Risk Classification



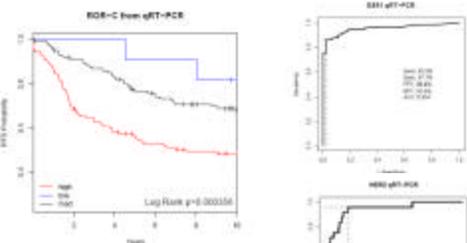
N= 710 untreated

100 re-sampled test sets from random split 2/3 train 1/3 test

Sizer analysis to identify linear relationship
Marron et al. Annals of Statistics, 2000

C-index: Harrell FE, Jr. et al. JAMA 1982

Performance of qRT-PCR Assay



N=279 test cases

Parker et al.,
JCO, In Press

12 weeks of paclitaxel
followed by 4 cycles
of FAC in 129 patients

Pharmacogenomic Predictor of Sensitivity to Preoperative Chemotherapy With Paclitaxel and Fluorouracil, Doxorubicin, and Cyclophosphamide in Breast Cancer

Joseph E. Peto, Erik Anderson, W. Fraser Symons, Yvonne Valero, Michel Ibrahim, James G. Mills, David Rosen, Richard L. Theriault, Anne T. Basler, Peter J. Duggan, Roman Rouzou, Hilar Terzaghi, Jeffrey S. Ross, Lawrence Valicenti, Glenn S. Gorman, Richard D. Harshbarger, and Lynn Piner

ABSTRACT

Purpose: We developed a multigene predictor of pathologic complete response (pCR) to preoperative weekly paclitaxel and fluorouracil/doxorubicin/cyclophosphamide (FAC) chemotherapy and assessed its predictive accuracy on independent cases.

Patients and Methods: One hundred thirty-three patients with stage I-III breast cancer were included. Pre-treatment gene expression profiling was performed with algorithmic microarrays on five-weekly aspirate specimens. We developed predictors of pCR from 82 genes and assessed accuracy on 51 independent cases.

Results: Overall pCR rate was 26% in both cohorts. In the training set, 56 probes were identified as differentially expressed between pCR versus non-pCR disease, at a false discovery rate of 1%. We examined the performance of 780 distinct molecular sets of genes as predictor algorithms in full cross-validation. Thirty predictors performed equally well. A nominal best algorithm (net Diagnostic Linear Discriminant Analysis classifier) was selected for independent validation. It showed significantly higher sensitivity (82%, P <math>0.001 to that of a clinical predictor including age, grade, and estrogen receptor status. The negative predictive value (96%, P <math>0.001) and area under the curve (0.87, P <math>0.001) were nominally better but not statistically significant. The combination of genomic and clinical information yielded a predictor not significantly different from the genomic predictor alone. In 27 patients, 89% was reclassified to baseline with leading predictors that were 87% concordant.

T/FAC Neoadjuvant Response By PAM50 Subtype

The overall pCR rate in this study was 22%

T/FAC pathological complete response rates for PAM50 subtypes and the triple-negative classification

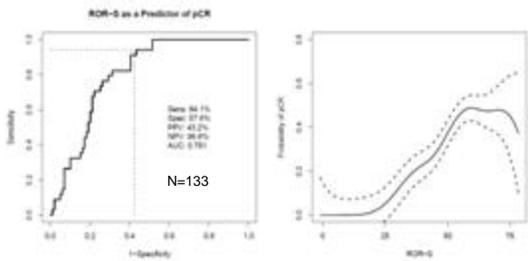
Classification	RD	pCR
Basal-like	11 (41%)	16 (59%)
HER2-enriched	17 (59%)	12 (41%)
LumA	36 (100%)	0 (0%)
LumB	22 (82%)	5 (18%)
Normal-like	13 (93%)	1 (7%)
Triple Negative	13 (50%)	13 (50%)
Any Positive	82 (80%)	20 (20%)
Triple Negative/Basal	6 (35%)	11 (65%)
Triple Negative/Non-Basal	7 (78%)	2 (22%)
Non-Triple Negative/Basal	4 (50%)	4 (50%)
Non-Triple Negative/Non-Basal	78 (83%)	16 (17%)

*Percentages are calculated by the total per classification

Parker et al., JCO, In Press and Presented at ASCO 2008, Parker et al., Abstract 11008

Data from 83 patients was earlier published aRouzier et al., Clinical Cancer Research 11, 5678-85 (2005)

T/FAC Response By ROR-S



Hess et al., 2006 (n=51)
Sens: 92% PPV: 52%
Spec: 71% NPV: 96%

The Effect on Tumor Response of Adding Sequential Preoperative Docetaxel to Preoperative Doxorubicin and Cyclophosphamide: Preliminary Results From National Surgical Adjuvant Breast and Bowel Project Protocol B-27

By Mary D. Donnan, Robert Anderson, Ann Shown, Roy Teich, William F. Barlow, Ronald Fisher, Richard Gelber, and Thomas W. Whitworth

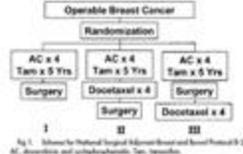
Purpose: The National Surgical Adjuvant Breast and Bowel Project Protocol B-27 was designed to determine the effect of adding docetaxel after four cycles of preoperative doxorubicin and cyclophosphamide (AC) on clinical and pathologic response rates and on disease-free and overall survival of women with operable breast cancer.

Patients and Methods: Women (N = 3,411) with operable primary breast cancer were randomly assigned by computer either four cycles of preoperative AC followed by surgery (group A), or four cycles of AC followed by four cycles of AC followed by surgery and then four cycles of docetaxel (group B). Clinical and pathologic tumor response to preoperative therapy were compared.

Results: More tumor size (5.1 cm) and other less characteristic, were usually histologically similar to those in patients. Death or toxicity rates observed in 10.0% of 1,000 patients during docetaxel treatment, and in 10.4% of 1,000 patients during docetaxel treatment. Compared to preoperative AC alone, preoperative AC followed by docetaxel increased the clinical complete response rate (88.1% v 83.0%; P = .002), the overall clinical response rate (93.0% v 87.0%; P = .002), the pathologic complete response rate (13.6% v 10.1%; P = .001), and the proportion of patients with negative axilla (46.0% v 40.0%; P = .001). Pathologic primary breast tumor response into a significant predictor of pathologic nodal status (P = .001).

Conclusion: The addition of four cycles of preoperative docetaxel after four cycles of preoperative AC significantly increased clinical and pathologic response rates for operable breast cancer.

J Clin Oncol 23:4047-4054. © 2005 by American Society of Clinical Oncology



All patients, All Arms results

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All Patients

	pCR	CR	ER	neg ER	pos	pCR	rate
Docet	14	47	66	98	8	230	0.230
AC	4	18	24	44	5	174	0.174
Lum A	4	15	21	40	4	161	0.161
Lum B	0	32	45	60	4	212	0.212
Norm	10	24	4	210	333	1313	

High Confidence subset (<=0.05)

	pCR	CR	ER	neg ER	pos	pCR	rate
Docet	13	41	52	81	8	207	0.207
AC	3	18	24	40	5	160	0.160
Lum A	3	15	21	39	4	156	0.156
Lum B	0	31	44	59	4	208	0.208
Norm	9	23	3	207	328	1285	

Moderate confidence (>0.05)

	pCR	CR	ER	neg ER	pos	pCR	rate
Docet	15	48	62	99	8	233	0.233
AC	1	28	35	44	5	164	0.164
Lum A	2	16	23	41	5	163	0.163
Lum B	0	34	47	61	4	210	0.210
Norm	9	26	3	208	333	1313	

ACT vs. AC results

All patients according to ER by central IHC (Data PharmD)

	ACT	AC
ER neg	1/11	1/58 (0.17)
ER pos	1/65	9/140 (0.06)

over 95% confidence data only

	ACT	AC
Docet	0/24 (0.00)	0/41 (0.00)
ER2	1/7 (0.14)	1/22 (0.04)
LumA	1/18 (0.06)	1/37 (0.03)
LumB	0/9 (0.11)	2/29 (0.11)
Normal	0/47 (0.07)	1/3 (0.2)

Moderate confidence

	ACT	AC
Docet	0/24 (0.00)	0/41 (0.17)
ER2	1/12 (0.08)	1/35 (0.03)
LumA	0/10 (0.1)	1/37 (0.03)
LumB	0/12 (0.15)	1/45 (0.03)
Normal	0/12 (0.5)	1/13 (0.12)

Multigent Chemotherapy Response

Subtype	Pathologic Complete Response Rate				Overall Prevalence
	NSABP B-27	ISPY	Hess et al	All Studies	
Luminal A	0/26 (0%)	2/39 (5%)	0/36 (0%)	2/101 (2%)	27%
Luminal B	1/7 (13%)	4/26 (13%)	5/22 (19%)	10/55 (15%)	17%
Her2-enriched	1/10 (9%)	12/10 (53%)	12/17 (41%)	25/37 (40%)	16%
Basal-like	13/41 (24%)	15/29 (34%)	16/11 (59%)	44/81 (35%)	33%
Normal-like	3/1 (75%)	3/3 (50%)	1/13 (7%)	7/17 (29%)	6%
All Subjects	18/85 (17%)	36/107 (25%)	34/99 (26%)	88/291 (23%)	

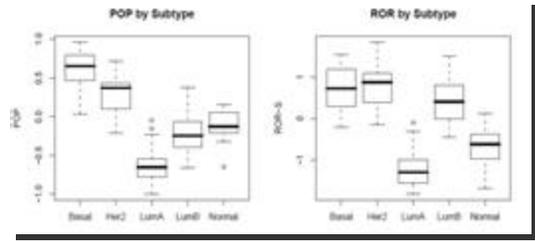
- Different multigent regimens
- NSABP B-27: AC or AC/T
 - Hess et al: T/FAC
 - ISPY:

Hess et al., JCO 2006
Beat et al., JCO 2003

Subtype Frequencies Across 13 Studies

	Luminal A	Luminal B	Her2-enriched	Basal-like	Normal-like	Total	Outcome	Platform
CALGB9840	17	36	21	30	8	112	OS, RFS	DASL
CALGB9342	24	12	12	20	1	69	OS, RFS	DASL
NSABP B-27	97	66	47	86	30	326	pCR, OS	Affymetrix U133+2
XENA	44	25	25	45	15	154	pCR	Agilent1x44k
US Oncology	28	17	17	29	7	98	pCR	Affymetrix U133A
ISPY	34	21	22	38	9	124	pCR, OS, RFS	Agilent1x44k
TAM	370	346	66	8	16	806	OS, DSS, RFS	qRT-PCR
van de Vijver et al.	89	70	53	53	30	295	OS, RFS	Agilent
hshina et al.	91	67	36	30	21	245	RFS	Affymetrix U133A
Hess et al.	39	22	29	31	12	133	pCR	Affymetrix U133A
Wang et al.	78	70	45	60	33	286	RFS	Affymetrix U133A
Loi et al.	145	135	44	37	33	394	RFS	Affymetrix U133A & U133+2
UNC	91	65	61	117	44	378	OS, RFS	Agilent
Total	1147	952	478	584	259	3420		
Total	33%	27%	14%	18%	8%			

Subtype Distribution of POP Differs from ROR-S



Genomic Assay Conclusions

1. The subgroup of ER+ and node-negative patients are the group for whom prognostic gene expression assays are of the most value.
2. Genomic assays like the PAM50 Intrinsic Subtypes, OncotypeDX, MammaPrint, and the Theros Breast Cancer Index, are providing new and valuable information that is not provided by the standard clinical variables.
3. The best treatment plan is guided by a combination of conventional clinical assays (ER status, node status and tumor size), and genomic biomarkers, and the two are complementary.
4. Genetic biomarkers (like CYP2D6 for tamoxifen responsiveness) are important and may also come into standard use.

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